BIOPROCESS DEVELOPMENT FOR THE BIOLOGICAL REMOVAL OF IRON FROM SUBSURFACE DRINKING WATER IN UTTARAKHAND

SYNOPSIS SUBMITTED

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BIOTECHNOLOGY

BY

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DECLARATION

I hereby declare that the synopsis entitled **"Bioprocess development for the biological removal of iron from subsurface drinking water in Uttarakhand."** is my original work and it has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles.

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Background

According to United Nations Department of Economic and Social Affairs, Population Division, around 7.4 billion people live on earth (Thapa, 2013). As the population is increasing according to WHO approximately 22% of the world population do not have accessibility to safe and clean drinking water. The main aim of water treatment is to reduce the hazard from physical, chemical and biological contaminants to the levels which are acceptable. Thus, ensuring that consumers get water of high aesthetic quality i.e. color, odor, taste and clearness are not offensive (Payment 2009; Marsidi et al. 2018).

In India presence of excess iron in water resources is an emerging issue especially in northern and eastern India (Srikanth 2009; Final Report 2006). Most of the North and Eastern states are having problems related to high iron content in water, regions like Meghalaya, Mizoram, and Manipur have approx. 100% of iron contamination (Final Report 2006). The report prepared by the Central Ground Water Board states that iron content in the ground waters of the following states - Andhra Pradesh, Assam, Bihar, Chhattisgarh, Goa, Gujarat, Haryana, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal and Andaman & Nicobar is found to be greater than 1.0 mg/l (CGWB 2010a). Several studies have also pointed out the contamination of iron in Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura of the North Eastern States (Srikanth 2009; Singh et al. 2008; Singh 2004). Few reports also suggest that ground water samples of West Bengal and Bihar have high levels of iron content in them (Lalwani 2004). States like Orissa, West Bengal, Bihar and the North Eastern States have implemented iron treatment plant for the removal of iron from the water (Final Report 2006).

In Uttarakhand the major concern which restricting the usage of sub-surface ground water is its excess iron contamination. Due to the presence of iron in sub-surface ground waters, the drinking water supply department of the state is highly concerned .This issue can be resolved by using various techniques and processes to treat iron before utilization of water but these methodologies failed because of different reasons like use of harmful chemicals, lack of availability of local regeneration materials, remote areas in hilly regions and waste disposal systems etc. but we still need better, safer, and cheaper techniques, the search is on and will always. The major population of the state is still, lacks, to have safe and clean drinking water, even after investments and extensive efforts with respect to elevated levels of Iron, in

rural as well as urban areas because of lack of proper engineering applications, difficult geographically hilly terrain, required level of S & T interventions and especially, geogenic reasons. The identification and management of water pollution is very important along with the understanding of science of underground iron pollution. Majority of the pockets, the Uttarakhand koop's (indigenous hand pumps) developed by UJS (Uttarakhand Jal Sansthan) for providing quality drinking water in hilly areas and villages which are on hill tops are no longer giving the good quality of drinking water supply to the local mass population and still having water which is red coloured. Sadly because of unavailability of alternative resources of drinking water, people are bound to use the same heavily iron polluted water. Further, in few areas, hand pumps using ground and recharge water of sub-surface level are also not providing water of good quality because of high iron content, thus making it unsuitable for drinking and other domestic purposes.

Table1: Undesirable Presence of Iron in Drinking Water of Uttarakhand

S. N.	Name of District	Values	Reference
1.	Dehradun	0.02 to 2.35 mg/L	Siddiqui et al. 2006
		0.081 to 7.046 mg/l (pre-monsoon) 0.057 to 6.810 mg/l (post-monsoon)	Jain 2004
2.	Haridwar	7.540 mg/l (raw) 7.776 mg/l (supply)	Singh et al. 2010
		11 Water quality affected habitations w.r.t. Iron	IMIS, 2010
3.	Almora	1.96 mg/L	Kansal et al. 2008
4.	Nainital	0.040 to 1.479 mg/l (pre-monsoon) 0.054 to 1.295 mg/l (post-monsoon)	Jain et al. 2010
		8 to 113 mg/l	CGWB 2010b
5.	Udham Singh Nagar	0.12 to 3.0 mg/l	CGWB 2010
		0.06 to 2.08 mg/L	Banerjee et al. 2009

Pollution of aquatic environment due to heavy metals is becoming an emerging issue because they cannot be biodegraded easily. Age, gender, chemical species, route of exposure, nutrition of exposed individuals, genetics etc. are some of the factors on which toxicity of heavy metals depends (Tchounwou et al. 2012.). Due to leaching of metals from industrial effluents, contaminated soils and water reserves, the metals have made their way into the food chain. It is absolutely necessary to eliminate these poisonous heavy metals from the effluents before their disposal, to prevent any health related risks. Major causes of heavy

metal contamination involve solid wastes from animals, urban industrial aerosols, industrial and agricultural chemicals mining activities. Heavy metals also make their way into the water supply system from industrial effluents or from acid rains which breaches down soils and rocks, discharging heavy metals into groundwater, streams, and lakes (Alluri et al. 2007; Harith et al. 2011; Khedr et al. 2016). Iron (Fe) induces aesthetic, and operating problems when they accumulate in water. In the disinfection process these metals utilize chlorine and encourage bio-fouling and microbiologically produced corrosion in water supply systems. The main source of drinking water in the entire world is ground water.

Iron is the most common component of ground water, it is ranked four among the most abundant element and ranks second among the most abundant metal found in earth's crust. (Silver 1993; WHO 1996). Moreover it is very unacceptable because it leads to some aesthetic and operational issues together with discoloration, bad taste, staining, and accumulation in distribution systems, and occurrence of high turbidity. Traditional treatment processes (sedimentation, precipitation, filtration and electro coagulation) are effective in the removal of Mn and Fe ions, but there are certain drawbacks associated with them in terms of their efficiency, space requirements, stability, produce large volumes of sludge and treatment capacity, thus it increases the operational and maintenance costs (Pramanik et al. 2012). Based on taste and other inconvenient concerns, the World Health Organization (WHO) advises that 0.3 mg/l or less should be the iron concentration in drinking water (WHO 1996). Thus, if concentration of iron in water is higher than the given standards, then it must be eliminated before utilizing it for drinking purposes. If water remains untreated then it can create problems for consumers and municipality department that supplies drinking water to the population.

By keeping in mind the issues behind the failure of the methods employed so far, a distinct biotechnological method has been analysed for the utilisation in field for the remediation or elimination of iron from sub-surface drinking water resources. The new biotechnological approaches i.e. bioremediation and bio beneficiation with the help of microbes metabolizing metals has been accepted widely because they are eco-friendly and cost-effective. Microbes have ability to take up the metals actively (bioaccumulation) and/or passively (bio sorption) are quite effective in the elimination of metal ions from a contaminated solution mixture in economical and environmental friendly fashion. Distinct species of protozoa, bacteria and fungi have been identified which show resistance to excessive concentrations of heavy metals in water. From several studies it has been shown that these microbial consortia are versatile and have potential for remediation of heavy metals from contaminated water, thus they hold high value for environmental clean-up. Biological drinking water treatment methodologies basically rely on the

development of bacterial communities that have potential of mediating oxidation-reduction reactions involving at most one target contaminant

The biological methods used for iron removal are green technologies which are less expensive as compared to conventional methodologies (Isaeva et al. 2011; Olanczuk-Neyman et al. 2000).The bioremediation strategies usually involve biological approaches in which microbes are utilized for the remediation of various kinds of contaminants from water. In the last 20 years the biological strategy based biofilm technique is used as an alternative treatment to physico-chemical approaches. Some researchers have implemented trickling filters to remove the iron from water. Pilot scale trickling filters were constructed to remove iron from drinking water. The combo of physico-chemical oxidation and biological oxidation is able to remove the filter depth by 40% (Tekerlekopoulou et al. 2007). The biological treatment approaches are usually based on the microbial activities for the oxidation of inorganic and organic constituents (Madoni 2011). Dhokpande and Kaware (2013) reported that biological methods have upto 90% efficiency in the remediation of heavy metals. This method is obviously better than chemical approaches as there are no chemicals involved, further it has low maintenance and operational cost (Hasan et al. 2009). The recent available biological methods involve the application of BAF (Biologically Aerated Filters) system as it offers several advantages. The BAF system is able to remove physical as well as chemical pollutants. It has been reported that (Fu et al. 2011) around 90% - 99% of Fe and Mn ions can be removed simultaneously using BAF systems. It was also observed that approximately around 98% of Fe ions can be removed efficiently by using BAF's or Biological Aerated Filters have several advantages as space requirement is low, suspended solids are also removed, generates low amount of sludge, further these are easy to handle and construct, and also have potential to remove excessive organic load (Mann et al. 1997).

The iron-oxidizing bacterium which oxidizes iron, have ability to remove iron from different types of water resources. These microbes oxidize the Fe^{2+} to Fe^{3+} iron biologically and remove it from water. This process is simple and does not require chemical oxidants, thus reducing the operational cost of the process. Ever since the discovery of iron bacteria, the approaches to use them for the removal of iron from contaminated drinking water has been exploited vigorously by the water processing engineers, the exact mechanism which involves the removal of iron from water by the iron metabolizing bacteria is still not completely known (Daniel et al. 2009). The most notable quality of iron-oxidizing bacteria is that they oxidize ferrous iron to ferric iron biologically; this process is very much comparable to physico-chemical reactions. The iron-oxidizing bacteria obtain their necessary energy demands particularly through a chemolitho-trophic process (Sierring et al. 1996). This process is facilitated by an enzyme which carries

the oxidation of Fe ion and simultaneously carbon dioxide is fixed into essential nutrients which are assimilated by the iron-oxidizing bacteria. Different kinds of bacteria which oxidize iron may be included in iron contaminated water treatment processes but in entire case, the biological oxidation reaction is taken as a catalytic process by nature, which causes the oxidation of insoluble ferric hydroxides quickly, and is found to be much better than the other treatment processes which form precipitates. (Soogard et al. 2001).

Those Bacteria which oxidize iron/manganese have been identified for their potential to precipitate manganese or iron oxides in structures present outside their cells. (Sogaard et al. 2000). In an oxidation process in *Leptothrix discophora,* they reacts with the soluble iron or manganese, Fe^{+2}or Mn^{+2}, which converts the iron or manganese in an insoluble form, Fe^{+3} or Mn^{+4} (Sierring et al. 1996). Biological and physico-chemical based treatment techniques are available for the removal of iron or manganese. The benefits of biological treatments as compared to the traditional physico-chemical techniques can be outlined as follows: chemicals are not used, give higher filtration rates, the probability of utilizing direct filtration and reduced maintenance and operation cost (Mouchet 1992). The micro-organisms accountable for this phenomenon belongs to the following generas- *Gallionella, Leptothrix, Crenothrix, Clonothrix, Siderocpasa, Sphaerotilus, Ferrobacillus* and *Sideromonas* (Wolf 1964; Degremont 1991; Corstjens 1993). Majority of these iron-oxidising bacteria are extended world-wide and are commonly found in ground-water, hypolimnion of lakes or impoundments, ponds, sedimentary deposits and soil. Bacterial oxidations takes place by two mechanisms which are reported (Czekalla et al. 1985; Bourgine et al. 1994; Sharma et al, 2005): (i) intracellular oxidation which takes place by the autotrophic bacteria, enzymatically. *(Gallionella and Leptothrix ochracea*), (ii) extracellular oxidation takes place by the catalytic activity of polymers which are excreted by iron bacteria (*Gallionella, Sphaerotilus, Leptothrix, Clonothrix, Crenothrix and Siderocapsa*).

Some reports regarding the ground water quality for drinking purpose have been reported (Jain et al. 2010; Ziauddin et al. 2006). Similarly, data about the bio-monitoring of drinking water is either not reported or very little information is available (CPCB 1999 & 1995). Few relevant references (Sood et al. 2008; Semwal et al. 2006; Seth et al. 2014; Dalai et al. 2014; Siwila et al. 2017) which belong to analysis of various sources involving lakes and rivers of Uttarakhand have also been widely published and cited.

For the remediation of the excess iron contamination, a number of different treatment techniques are being utilized. An effective treatment strategy is required to reduce the high levels of iron and to provide safe and clean drinking water. A number of approaches have been reported. There are different forms of

iron present in water. These various forms can be categorized into various types based on solubility, mineral form and chemical nature of iron. (Khatri et al. 2017).The treatment methodologies initially rely on the different existing forms of iron. Any one single technique is not suitable for the elimination of all forms of iron (Colter et al. 2006; Daniel et al. 2009; Skimpton 2014; Siwila et al. 2017). EPA (Environmental Protection Agency) and other different water agencies have suggested the five initial treatment processes for the elimination of high iron content in drinking water (EPA 2010). The alternative processes for iron elimination are discussed below –

Ion exchange water softener: Ion exchange is most usual method that can eliminate even the small amounts of ferrous iron from 2 to 5 mg/l. In this process iron particles get exchanged with the Na or K on the ion exchange medium. But this is not a sensible method because it involves addition of ions in the water (Soogard et al. 2000; AGWT 2008; Heffron et al. 2016).

Aeration with filtration: When oxygen is present, dissolved Fe get transformed into solid particles of ferric iron. In this process, aerator mixes the air which is oxygen rich with water and changes soluble iron into insoluble iron, which gets filtered afterwards. This method found to be useless for iron concentrations which are higher than 25 mg/l. (Herman 1996; Sharma et al. 2005; Kasim et al. 2015).

Chemical oxidation with filtration: By the utilization of chemicals like chlorine, potassium permanganate or hydrogen peroxide, the ferrous iron is oxidized into insoluble ferric form and then filtered out as solid particles from the processed water. This technique is suitable for the elimination of iron up to 10 mg/l in water (WDNR 2010).

Oxidizing filters: These types of systems can be utilized to treat iron containing water with ion exchange materials like manganese greensand or manufactured zeolite smeared with manganese oxide, which soaks up the dissolved iron. It has potential of eliminating up to 99% of the Fe and is efficient in removal of the iron content up to 15 mg/l (IDPH 1999; Sharma et al. 2005; Barlokova et al. 2009; Ling 2011; Zhao et al. 2015).

Phosphate treatment: This technique is very economical to eliminate the low iron levels upto 3 mg/l. Phosphate compounds gets attached to the iron and block them from slipping out of the solution. Water contains a metallic taste, after this process. (Colter et al. 2006).

Iron although itself is not very harmful or poisonous element, but causes reddish-brown or black stains on household materials and clothes (BIS 1991; WHO 1996; Jain et al. 2010). The Environmental Protection Agency (EPA 2010) guidelines for drinking water have also assessed it as a secondary pollutant which cause unpleasant taste, odour, colour, corrosion, foaming or staining but does not have any direct effect on health (IDPH 1999). In groundwater and surface water the concentration of iron ranges from varies from 3 to 4 mg/L to 15 mg/L. In West Bengal its concentration ranges from 2-10mg/l (Sengupta et al. 2001). Near the Fazalpur industrial area, in Ganga River, in Moradabad district of Uttar Pradesh, India the iron concentrations up to 6mg/l have been reported (Kumar et al. 217). In Assam the ground water is highly contaminated and have high levels of iron content (Mahanta et al. 2004; Houben 2003). The permissible limit for the purpose of drinking is 0.3mg/l of water (Iron in drinking water 1996, Khatri 2016). Normally, contamination of iron above this concentration, in drinking water may does not have any harmful effects. But regular intake of such high levels of iron may cause a condition called "iron overload" (Zheng et al. 2017). High levels of iron intake may result in the detriment of hematopoesis by destroying the micro-environment required for hematopoiesis, as well as the progenitor cells. When the iron overload is remains untreated, it may result into hemochromatosis, which causes damages to various organs of the body (Hartmann et al. 2013; Prus et al. 2010). Primary symptoms in the beginning include fatigue, weight loss, joint pain, disorders of eye like choroiditis, retinitis, and conjunctivitis, heart problems are few health related concerns that occur due to presence of elevated levels of iron in water (Alimohammad et al. 2017). Apart from these problems the metal imparts the metallic taste, an odor, and red colour to the water when present in excess concentrations. Further, the excess concentration of iron may also found to act as a substrate for some bacteria (Michalakos et al.1997). The unpleasant taste of water is due to presence of soluble ferrous iron. However, when it is mixed with tea, coffee and other beverages, it gives an inky, black colour and an unsuitable taste. Fe is an essential requirement for the human body system but its excessive intake in high amount may cause several health related issues like diphasic shock, anorexia, diarrhea, metabolic acidosis, congestion of spleen, gastrointestinal tract, brain, thymus and death (Namdeo et al.2008; Yavuz et al. 2005). Prolonged presence of iron in drinking water can be a causal factor of serious liver disease (Idoko 2010). Moreover, excessive iron content is linked with the severe brain related issues. Alzheimer's, epilepsy, Parkinson's and autism are serious diseases, which happen by over-loading of iron. Hence, without being aware of injurious effects of profused iron content, people are neglecting the concerns of iron overload (Barfield 2009).

By the support of points mentioned above, the identification of iron metal is becoming compulsory in the environmental samples like water. Further , keeping in mind the related health issues and biological concerns emerging out due to over dose/ presence (and hence, high intake) of iron in drinking water, so it

is suitable to find and create new treatment methodologies which have potential of remediation of unwanted iron from sub-surface water, particularly through the biotechnological approaches, like utilization of suitable microbial consortium in eliminating/reducing iron contamination in sub-surface water is much better strategy for which detailed research is necessary. (Tyrell et al. 1998; Gage et al. 2001; Sharma et al. 2005; Soogard et al. 2009; Hassan et al. 2015; Khedr et al. 2016; Siwila et al. 2017). In addition, the techniques to be utilized in the process should be economical as well as environmental friendly.

The proposal is been actively associated in evaluating the water characteristics at state level as well as investigating and identification of different microbial strains which have ability of producing various metabolites and capable of decomposition of complex organic compounds that are present in the water. Under the proposal, it is planned to isolate and determine series of different bacterial strains, formulation of bacterial consortia, characterization of the active enzyme(s) associated in the removal/binding/absorption of organic compounds as well as the metal ions especially iron via bioaccumulations/bio absorption phenomenon. Elaborate evaluation of bio-absorptive and bio-accumulative processes is required on both physical and chemical parameters, for an effective treatment strategy. Biological drinking water treatment techniques relies on the development of bacterial communities that have potential of facilitating oxidation-reduction reactions having at most one target contaminant utilising an organic or an inorganic electron donor species. Further, outlining of the various bioreactors and performing laboratory scale studies for drinking water treatment, employing the experimental bacterial strains and bio-filters/ bio-remediating processes for iron elimination would be the main focus. The entire study will serve as a pathway for application of the immensely valuable bacterial isolates in cleaning up of water resources using biotechnological tools.

Objectives of the study-

Water is an essential part of our life, our fresh water resources are our main sources of water supply. But the most well-known issue of water resources of different regions in India is the elevated levels of iron content in the water samples including eastern and northern India. In Uttarakhand, because of long-term exposure of sub-surface water with rocks and minerals i.e. geogenic pollution is the main cause of iron content in drinking water. The aim of the proposed research is to have an eco-friendly as well as a sustainable approach and awareness of consumers with respect to drinking water pollutants involving iron in rural pockets of hilly areas, and thereby enhancing overall health.

The major objectives of the overall studies are -

- Search for sustainable, better, cheaper and safer technique for removal of iron from subsurface water.
- Technical evaluation of different biotechnological parameters for the iron removal from subsurface water of the designated site of study.
- Designing of Bioreactors for the iron removal/treatment strategy.
- Development of bioprocess at laboratory scale.
- Economic evaluation of the method developed in comparison with other methods used for the same purpose.

Hypothesis

Supply of the safe and clean drinking water is big concern in Uttarakhand. Major population of the state does not have the access to quality drinking water even after the huge investments and massive efforts, with respect to higher levels of iron contamination in rural as well as urban areas. The hypothesis of the proposed issue includes studies based on analysis and reduction/elimination of iron from different water resources by implementing efficient bio-remediation techniques using appropriate microbial consortia. The so far efforts made by the different agencies have not exhibited positive results and all the imported or market technologies utilized are turned out to be disappointment because of different reasons involving usage of chemicals, absence of regional availability of regeneration material, waste dumping and remote locations in hilly areas etc. The proposed scheme could be utilized efficiently for meeting out drinking and domestic necessity of good quality of water as per BIS.

Key Questions

- If the resulting method ultimately provides an efficient biotechnological treatment process for removal of total iron content.
- If the development of the consortium of microbes in suitable designed bioreactor is able to reduce the iron load.
- If the enhanced iron load is changing the physico-chemical parameters of the river system.

Methodology

1. During the first six months of work literature survey will be done for upgrading the knowledge.
2. Water samples from 50 hand pumps and 50 Uttaranchal Koop's of Haridwar district will be collected.

3. Selection of water samples of 05 hand pumps and 05 Uttaranchal Koops i.e. from 10 sites in Haridwar district containing highest quantity of iron for further treatment will be carried out.
4. Physico-Chemical analyses/ assessment of water samples from the sites of high Iron content.
5. Utilisation of low cost materials like Kaolinite, bentonite, lignite and pumice will be investigated to assess their capability to remove iron.
6. Collection and screening of the soil samples from the area of high metal ion contents and the pristine areas of the Uttarakhand for the isolation of bacterial strains capable of biosorption/ bioaccumulation of metal ions will be initiated with the raw water collected from the designated 10 identified sites (villages/ cities) of Haridwar district of Uttarakhand.
7. The overall methodology will involve the following steps for the bioabsorption/bioaccumulation of metal ions from water:

I. Analysis of subsurface water samples for iron form and quantity.

II. Bioleaching and secondary metabolite studies of high iron containing water will be undertaken for identification and characterization of type of iron present and for additional molecules/ biomolecules/ microbes produced due to activated behaviour of iron in water.

III. Screening and isolation of Microbial strains (available and new) against different raw water samples

a. Sequential enrichment techniques
b. Plate Culture Assay
c. Liquid Culture Assay
d. Batch Culture Enrichment

IV. Optimization of parameters for Bio sorption of metal ions

a) Organism growth rate
b) pH
c) Temperature
d) Nutrients
e) Bio absorption rate
f) Hydraulic retention time (HRT)

V. Immobilization of the characterized soil microbes on different media, gravels, sand, coarse sand, bentonite, lignite etc.

i. Characterization and formulation of soil media for the treatment of raw water

a. Composition
b. Physicochemical properties

c. Determination of components bed length
d. Optimization of packing materials containing microbes
e. Half-life of bacterial consortia in filter beds
f. Bio sorption capacity
g. Sludge generation
h. Recharging of the filter beds

ii. Designing of Bioreactors (biologically stabilized soil filters) in fixed bed/ fluidized bed/UASB

iii. Lab scale trials (5-10 L): Laboratory Process Development

Experiments and will comprise:

a. Inoculation
b. Retention Time
c. Bio absorption rate of iron

iv. Water quality analysis for testing efficacy of the method developed for quantitative removal of iron from subsurface.

v. Cost economics will then be calculated and will be compared with per cubic meter water production cost through other methods.

vi. Interpretation of the data and conclusions will then be drawn.

Expected Outcome

1. Water quality assessment and monitoring to be carried out under the proposed research will help to evaluate the nature and extent of pollution, control, efficiency of pollution control measures that are already in existence, and drawing the water quality trends and prioritizing pollution control efforts.
2. Restoration and maintenance of aquatic resources is an area of interest of using biotechnological measures focusing on public health.
3. The study will be highlighting the assessment and monitoring of the water quality done on larger perspective to ensure that it is restored at desired level intended for drinking and domestic use.
4. Biological iron removal systems could have the following advantages:

- Good outcome in eliminating iron through developed methodology.
- Simple method regarding implementation and operation.

- Eco-friendly, sustainable, and biological method with little chemicals associated with no or little generation of sludge as a consequence of iron elimination operation.
- Comparable production cost of one cubic meter of water by utilizing the prepared method than the production cost of water utilizing the other alternative methodologies.
- Field trial method of subsurface removal of iron to user for providing safe drinking water to mass population.
- These are self-sustaining systems, so low maintenance cost.
- Sensitization of consumers with respect to drinking water pollutants including iron in rural areas.
- Applicable to big cities for regional scale treatment and small communities for local-scale treatment.
- Can help in assessing the fitness of water for different purposes.

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